510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k181017

B. Purpose for Submission:

New device

C. Measurand:

Testosterone (free)

D. Type of Test:

Quantitative, Enzyme Immunoassay (EIA)

E. Applicant:

Monobind, Inc.

F. Proprietary and Established Names:

Free Testosterone AccuBind ELISA Test System

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
CDZ	Class I, reserved	21 CFR 862.1680 Testosterone test system	Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

See Indication(s) for use below.

2. Indication(s) for use:

   The Free Testosterone AccuBind ELISA Test System is an Enzyme Immunoassay (EIA) for the quantitative measurement of free testosterone in human serum. Measurement of free testosterone is used in the diagnosis and treatment of disorders involving the male sex hormones (androgens), including primary and secondary hypogonadism, impotence in males and in females; hirsutism (excessive hair) and virilization (masculination) due to tumors, polycystic ovaries and adrogenital syndromes.

3. Special conditions for use statement(s):

   For Prescription Use only.

4. Special instrument requirements:

   Microplate Reader with 450nm and 620nm wavelength absorbance capability.

I. Device Description:

The Free Testosterone AccuBind ELISA Test System consists of the following:

- Seven vials of 1 mL serum reference calibrators for Free Testosterone containing testosterone in human serum with preservative at concentrations of 0, 0.2, 1.0, 2.5, 7.5, 20, and 60 pg/mL.
- Three vials of 1 mL controls (one low, one medium, and one high level) containing free testosterone in human serum with preservative.
- Free Testosterone Enzyme Reagent: One vial of 6 mL Testosterone (Analog)-horseradish peroxidase (HRP) conjugate in a protein stabilizing matrix.
- Substrate A: one vial of tetramethylbenzidine (TMB) in buffer.
- Substrate B: one vial of hydrogen peroxide in buffer.
- One 96-well microplate coated with testosterone antibody and packaged in an aluminum bag with a drying agent.
- One vial containing hydrogen peroxide in buffer.
- One vial of 20 mL concentrated wash solution.
- One vial of 8 mL stop reaction solution.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   EiAsy Free Testosterone EIA

2. Predicate 510(k) number(s):

   k030730

3. Comparison with predicate

Similarities and Differences

Items	Candidate Device Free Testosterone AccuBind ELISA Test System (k181017)	Predicate Device EiAsy Free Testosterone EIA (k030730)
Intended Use	The direct quantitative determination of free testosterone by enzyme immunoassay in human serum.	Same
Antibody	Utilizes a highly specific rabbit polyclonal antibody at a low binding capacity.	Same
Sample Type	Human serum	Same
Test Principle	Competitive Enzyme Immunoassay	Same
Detection Instrument	Microplate Colorimeter Reader	Same
Microplate coating	Antibody coated microwell plate	Same
Calibrators	Seven vials containing testosterone in human serum with preservative.	Six vials containing testosterone in human serum with preservative.
Controls	Three vials containing testosterone in human serum.	Two vials containing testosterone in human serum.
Measuring range	0.11 - 60 pg/mL	0.018 - 60 pg/mL

K. Standard/Guidance Document Referenced (if applicable):

CLSI EP05-A3 Evaluation of Precision Performance of Quantitative Measurement Procedures (October 2014).

CLSI EP06-A Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach (April 2003).

CLSI EP17-A2 Evaluation of Detection Capacity for Clinical Laboratory Measurements Procedures (June 2012).

CLSI EP28-A3C Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory (October 2010).

CLSI EP07-A2 Interference Testing in Clinical Chemistry (November 2005).

CLSI EP25-A Evaluation of Stability of In-Vitro Diagnostic Reagents (September 2009).

L. Test Principle:

The Free Testosterone AccuBind ELISA test system uses a competitive enzyme immunoassay technology. The essential reagent includes an immobilized antibody, enzyme-antigen conjugate and a native antigen. Upon mixing the immobilized antibody, enzyme-antigen conjugate and a serum containing the free native antigen, a competitive reaction results between the native free antigen and the enzyme-antigen conjugate for a limited number of insolubilized binding sites. After equilibrium is attained, the antibody-coated fraction is separated from unbound antigen by decantation or aspiration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown sample can be ascertained. The serum calibrators are prepared in human serum matrix. The enzyme-antigen conjugate is labelled with horseradish peroxidase (HRP) and the substrate reagent contains tetramethylbenzidine (TMB), a blue color is produced. The reaction is stopped with addition of an acid and a yellow color is developed. The plate is read in a microtiter plate reader at 450nm.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

A study was performed by testing three levels of human serum pools and three levels of control material using three reagent lots. The samples were tested in duplicate, two times a day for a period of 20 days for a total of 80 measurements per sample. Each of the three lots of reagents produced similar precision results. The precision results from one representative lot are summarized in the table below:

		Within-Run		Total	
Sample	Mean (pg/mL)	SD	%CV	SD	%CV
Control 1	2.51	0.09	3.7%	0.20	7.8%
Control 2	10.98	0.40	3.6%	0.96	8.7%
Control 3	22.72	0.83	3.6%	2.18	9.6%
Serum 1	0.98	0.06	5.9%	0.12	12.4%
Serum 2	4.53	0.26	5.7%	0.36	8.0%
Serum 3	53.62	4.24	7.9%	4.32	8.1%

b. Linearity/assay reportable range:

A study was performed to evaluate the linearity of the Free Testosterone AccuBind ELISA Test System. Test samples were prepared by performing serial dilutions of a high human serum free testosterone pool with a low human serum free testosterone pool. The ten free testosterone concentrations tested were as follows: 0.11, 6.58, 13.05, 19.52, 25.99, 32.46, 45.40, 51.87, 58.34, and 64.81 pg/mL.

The samples were tested in replicates of 4. The following linearity regression equation was obtained:

$y= 1.0149x – 0.6028$, $R^2=0.9888$

The results of the linearity study support a measuring range of 0.11 - 60 pg/mL.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

The test system is traceable to a certified reference material, Cerrlian testosterone, T-037.

d. *Detection limit:*

Detection limit studies were performed in accordance with CLSI EP17 guideline.

A limit of blank (LoB) study was performed using three different blank samples that were measured using 3 reagent lots over 6 days to yield 144 measurements. The LoB was determined nonparametrically using the following equation: $[N_B(p/100)+0.5]$=result at position $[0.95*N_B=0.5]=P(1-\alpha)$. The LoB was determined to be largest result of the 3 reagent lots, 0.0295 pg/mL.

The limit of detection (LoD) study was performed by obtaining 10 measurements of 10 low-level samples across 3 different reagent lots and 2 analyzers. LoD was calculated using the following equation: LoD=LoB + Cv x SDs. The LoD was determined to be 0.0519 pg/mL.

Limit of Quantitation (LoQ): The test results from the LoD study were used to calculate total error. The goal for the total error was set as 0.05 pg/mL. The TE was lower than 0.05 pg/mL, therefore the sponsor claims that the LoQ is equal to the LoD, 0.0519 pg/mL.

Limit of Blank	Limit of Detection	Limit of Quantitation
0.0295 pg/mL	0.0519 pg/mL	0.0519 pg/mL

The sponsor's claimed measuring range is 0.11 - 60 pg/mL.

e. *Analytical specificity:*

Interference:

An interference study was performed following CLSI EP07-A2 guideline. Aliquots from pools of human serum with a free testosterone concentration of 7.916 pg/mL and 38.5 pg/mL were spiked with potentially interfering substances at one or more concentrations. The sponsor defines significant interference as > 10% bias.

The results are summarized in the following table:

Substance	Highest concentration at which no significant interference was observed
Acetaminophen	20 mg/dL
Acetylcysteine	150 mg/dL
Ascorbic Acid	6 mg/dL
Bilirubin, conjugated	15 mg/dL
Bilirubin, unconjugated	20 mg/dL
Biotin	100 ng/mL
Caffeine	6 mg/dL
Cholesterol	503 mg/dL
Creatine	30 mg/dL
Dextran	5000 mg/dL
Digoxin	6.1 ng/ mL
Doxycycline	50 mg/dL
Erythromycin	6 mg/dL
Gentamicin	1 mg/dL
Human mouse antibodies	440 ng/mL
Hemoglobin	500 mg/dL
Heparin	3 U/mL
Human serum albumin	2.5 g/dL
Ibuprofen	50 mg/dL
Immunoglobulin G	4 g/dL
Levodopa	20 mg/L
Lidocaine	1.2 mg/dL
Lipemia (glycerides)	1000 mg/dL
Methyldopa	20 mg/dL
Nicotine	0.1 mg/dL
Phenobarbital	15 mg/dL
Protein, total	10.5 g/dL
Rheumatoid factor	1110 IU/mL
Salicylic Acid	60 mg/dL
Sex hormone binding globulin	200 µg/mL
Triglycerides	900 mg/dL
Urea	500 mg/dL

Cross-Reactivity:
A cross-reactivity study was performed in accordance with CLSI EP07-A2 to evaluate whether various analytes or substances cross-react with the quantitation of free testosterone using the device. Aliquots from a pool of human serum with a free testosterone concentration of 7.408 pg/mL were spiked with substances at the concentrations listed in the table below. Cross-reactivity was determined using the following equation: (observed value - unspiked value)/ concentration of cross-reactant x 100%. The sponsor defines significant cross-reactivity as > 10% difference.

Substance	Concentration. of substance (ng/mL)	% Cross Reactivity
11-Deoxycortisol	1000	0.000%
11-KetoTestosterone	10	0.647%
11β-Hydroxytestosterone	100	0.065%
17α-ethynyl estradiol	1000	0.000%
17α-Estradiol	1000	0.000%
17β-Estradiol	100	0.000%
17-Hydroxypregnenolone	1000	0.000%
17-Hydroxprogesterone	10	0.000%
3-EstriolGluc	1000	0.000%
3-EstriolSul	1000	0.000%
3β-Androstanediol	500	0.000%
5α-Dihydrotestosterone	100	0.054%
Aldosterone	8000	0.000%
Amitriptyl HCI	1000	0.000%
Androsterone	1000	0.000%
Andronstenedione	1000	0.004%
Clomiphene Citrate	1000	0.000%
Corticosterone	1000	0.000%
Cortisone	1000	0.000%
Cortisol	1000	0.000%
Cyproterone acetate	1000	0.000%
D-5-Androstene-3β,17β-diol	1000	0.000%
Danazol	1000	0.000%
Dehydroepiandrosterone	100000	0.000%
Dehydroepiandrosterone Sulfate	1000	0.000%
Desogestrel	100	0.000%
Dexamethasone	1000	0.000%
Epitestosterone	1000	0.001%
Estriol	1000	0.000%
Estrone	1000	0.000%
Ethisterone	1000	0.000%
Ethynediol	1000	0.000%
Ethynediol diacetate	50	0.000%
Flunisolide	1000	0.000%
Fluoxymesterone	1000	0.000%
Lynestrol	1000	0.000%
Medroxyprogesterone acetate	1000	0.000%
Methyl Testosterone	100	0.000%
Mestranol	1000	0.000%
Norethindrone	50	0.000%
Norethindrone acetate	50	0.000%
Norgestimate	1000	0.000%

Substance	Concentration. of substance (ng/mL)	% Cross Reactivity
Norgestrel (Levonorgestrel)	50	0.000%
Norethynodrel	50	0.000%
Oxymetholone	100	0.000%
Prednisolone	1000	0.000%
Prednisone	800	0.000%
Progesterone	1000	0.000%
Salbutamol	1000	0.000%
Spironolactone	1000	0.000%
Stanozolol	1000	0.000%
Testosterone enanthate	10	0.000%
Testosterone SO4	1000	0.004%
Testosterone Propionate	1000	0.000%
Triamcinolone	50	0.000%

An additional study was performed to evaluate the cross-reactivity effects of testosterone cypionate and testosterone undecanoate. Aliquots from pool of human serum with a free testosterone concentration of 38.4 pg/mL were spiked with 12 ng/mL of testosterone cypionate and testosterone undecanoate. Cross-reactivity was determined using the following equation: observed value - unspiked value/ concentration of cross-reactant x100%. The sponsor defines significant cross-reactivity as > 10% difference. The results are summarized in the chart below:

Substance	Concentration of Substance (ng/mL)	% Cross Reactivity
Testosterone cypionate	12	0.000%
Testosterone undecanoate	12	0.000%

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

A method comparison study was performed by testing 137 (133 native and 4 spiked) human serum samples with the candidate and predicate devices. The test results on the candidate device ranged from 0.11-59.63 pg/mL. The following regression equation was obtained using Passing-Bablok analysis: y= 1.017x - 0.244, r= 0.997

b. Matrix comparison:

Not applicable. Serum is the only claimed sample type.

3. Clinical studies:

   a. Clinical Sensitivity:

   Not applicable.

   b. Clinical specificity:

   Not applicable.

   c. Other clinical supportive data (when a. and b. are not applicable):

   None.

4. Clinical cut-off:

   Not applicable.

5. Expected values/Reference range:

   A reference range study was performed by testing a total of 261 serum samples obtained from 130 females (aged 22-93 years), and 131 males (aged 22-89 years) on the candidate device. The subjects were not pregnant, not on hormone therapy, not taking contraceptives, glucocorticoids or corticosteroids; and had no history of thyroid, autoimmune, Cushing's, or Addison's disease. The resulting reference interval is summarized in the following table:

Cohort	N	95% Confidence Range (pg/ml)
Male, 20-39 yrs.	45	9.2-34.6
Male, 40-59 yrs.	43	6.1-30.3
Male, ≥60 yrs.	43	6.1-27.9
Female, 20-39 yrs.	44	0.2-6.1
Female, 40-59 yrs.	42	0.3-4.4
Female, ≥60 yrs.	44	0.5-3.4

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.